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## THREE NEW GREGARINES FROM MARINE CRUSTACEA \*

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The protozoan parasites described here were studied during the summers of 1913 and 1914 at the Biological Laboratory of the Brooklyn Institute, Cold Spring Harbor, L. I., and I wish to thank Dr. C. B. Davenport, the director, for kindly affording me the privileges of the laboratory.

All the hosts were found on the Sand Spit, a narrow peninsula half a mile long separating the outer and inner harbors. The area is thus geographically very restricted, and although the hosts are similar and the possibility of all of them being infected with the same parasite considered, yet there is abundant evidence to warrant regarding the three forms described below as separate species.

### *Frenzelina delphinia* nov. spec.

Figures 1 to 8

The host of this gregarine is the large white sand flea, *Talorchestia longicornis*, which is found in fine sand between tide marks. Fleas were taken from the eastern end of the Sand Spit, and also from locations along the road to Lloyd's Neck, from Huntington Beach, and from Northport Harbor; and hosts from all these localities were found to be infected.

The parasites were present in 30 per cent. of the 260 intestines examined, the number in an infection varying from one to ten in 75 per cent. of the hosts and from ten to 500 in the other 25 per cent.

The sporonts (Figs. 1 and 2<sup>1</sup>), which live free in the lumen of the upper part of the intestines, are small, the average of fifty large individuals being  $110\mu$  in length and  $60\mu$  in width. The largest individual seen measured  $115\mu$  in length and  $64\mu$  in width. The ratio of length of protomerite to total length of sporont is about 1:4; the ratio of width of protomerite to width of deutomerite about 1:1.5. A table of a few measurements is given at the end of this section.

The sporonts are biassociative when mature and the two members of an association do not differ materially in size; either the primate or the satellite may be a little longer or broader than the other. The sporonts are stout bodied, being less than twice as long as wide. The

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\* Contributions from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 60. Also from the Biological Laboratory, Cold Spring Harbor.

<sup>1</sup>For explanation of special terms used see description of Figure 1.

protomerite is cylindrical and broadly rounded in front and is about three fourths as high as wide. There is often a slight constriction at the septum which separates the protomerite and deutomerite. The deutomerite is doliform, two thirds as wide as long, widest through the middle portion, and terminates in a broadly rounded extremity. There is a small conspicuous papilla at the anterior end of the protomerite of the young sporonts which persists, although reduced in size, in the adults. In the satellite of an association it becomes a structure for the attachment of the two sporonts by projecting upward and forming a small indentation in the posterior end of the primite (Fig. 4). Hydrochloric acid, either dilute or concentrated, fails to dislodge the two sporonts, but sodium hydroxid or ammonium hydroxid, in solution, readily disassociates them.

The endocyte of the mature sporonts is light brown in color and in full-grown animals fairly dense, and the nucleus not visible. Young sporonts are less dense and the color paler. The satellite of an association is generally less dense than the primite, and for this reason its nucleus is often visible *in vivo* when that of the dense primite is not. The protomerite of a sporont is slightly less dense than the deutomerite and contains fewer protoplasmic granules; the granules are slightly larger than those in the deutomerite and the endocyte paler in color. When the host has just been taken and has probably fed the night before, full grown sporonts are dense; when, however, the fleas have been kept in damp sand for a few days without food, the parasites are likewise deprived of nourishment and become pale in color and the number of protoplasmic granules present, hence the density, is greatly reduced. The addition of a drop of iodine solution renders these pale parasites visible but of course kills them.

The nucleus of the sporonts is generally visible *in vivo* only as a lighter indefinite area although in young and starved sporonts it is often visible. It is large and spherical and contains one or two large homogeneous karyosomes. The epicyte is thin and fragile and is marked with longitudinal striations only visible under an oil immersion lens.

Cross sections made of the intestine of the host reveal the fact that development is intercellular as in the family of the Stenophoridae. This is the first genus of the family Gregarinidae which has been found to develop in this manner, the other members possessing an epimerite which alone is attached to the host cell, the remainder of the trophozoite projecting into the lumen of the intestine. In the species here described, the whole trophozoite is embedded in the epithelium (Figs. 3 and 6). When stained with Delafield's hematoxylin, the cells of the epithelium become purplish-blue, while the parasites stain less deeply and the color is a clear homogeneous blue with a darker blue nucleus.

The animals are capable of movements both of bending and of gliding progression. The sporonts are able to move through a narrow place in the manner employed by an amoeba. When the host intestine is first opened, a mosaic of inert distorted individuals or associations lying near the epithelium is often revealed; when, however, water or normal salt solution is added, the smooth, regular contour is quickly restored. Normal salt solution stimulates movement and sporonts often remain alive and motile for an hour and a half. Sea water has the same effect. As motion tends to be retarded at the end of this time, weak tannic acid solution was added in a few instances and caused considerable acceleration of movement; plasmolysis, however, occurred inside of another half hour and the animals died.

That transparent threads of mucus are present at the posterior end of the body was frequently attested by the fact that the animals were able to carry with them in their movements large or small masses of débris at a distance behind the body often as great as the length of the animal itself. A mass twenty-five times the volume of the gregarine itself was in one instance observed being carried along.

Cyst formation was observed in material from the host intestine from its incipency until rotation ceased. The adult sporonts free in the intestine which are ready for cyst formation become thickened and shortened, motion becomes sluggish, movement of progression ceases and that of bending becomes more active (Fig. 5). Concomitant with the revolving motion there occurs a deposition of gelatinous threads exuded from the body in fine concentric layers around the revolving mass. The sporonts become a spherical mass and the threads form a thick cyst wall. The rotating mass passes from the mid intestine to the rectum and ceases motion (Fig. 7). It begins here its development by the loss of the wall separating the two sporonts and the disintegration of the two sporont nuclei. The protoplasm of the cyst collects in masses and on the periphery of each protoplasmic mass are formed spherical protuberances, the gametids. The cyst is now expelled with the feces. Cysts when expelled are somewhat opaque, tan in color, and average  $80\mu$  in diameter, including the cyst wall.

It is difficult to effect cyst development to completion by artificial methods. Marine bacteria seem to be virulent and to cause putrefaction or otherwise stop development in the early stages. Cysts were kept in the damp chamber in normal salt solution and a few yielded gametes upon being crushed when 24 hours old. The gametes were stained with safranin; they were large and nearly spherical, and no difference in size was observed in those from the same cyst. Some of the gametes, however, showed large deeply staining nuclei and others smaller nuclei which stained less deeply, probably because of their reduced chromatin

content. A difference in the staining reaction was also noted in the two sporonts in a cyst of about eighteen hours, the one sporont mass staining more deeply than the other.

A very few cysts developed to completion and dehiscid by simple rupture in 35 hours, but no well formed spores were present.

*Orchestia agilis*, the smaller very common sand flea, was found in one instance to be infected with three nonassociative sporonts which agree in size and shape with the species described above. This flea was also heavily parasitized with an infusorian. *Orchestia grillus* from the roots of the eel grass (*Juncus palustris*) was not found to be infected with gregarines.

The genus *Frenzelina* (Léger and Duboscq, 1909) has not hitherto been reported from the United States and only seven species have been described. The species described above is placed in this genus because (1) the sporonts are biassociative, (2) the cysts dehiscid by simple rupture, (3) development is intercellular, (4) the apex of the protomerite is slightly papillated, (5) the parasite inhabits the intestine of a crustacean.

I wish to designate the species, of which no previous record is found, *Frenzelina delphinia*.

The fact that development is intercellular was not determined by Léger and Duboscq and this important fact should be added to the features which characterize the genus *Frenzelina*.

A table of typical measurements, in microns, follows:

Total length association.....	215	215	210
Primites:			
Total length sporont.....	115	110	112
Length protomerite .....	20	24	27
Length deutomerite .....	95	86	93
Width protomerite .....	39	35	43
Width deutomerite .....	62	66	63
Ratio length protomerite: length deutomerite .....	1:4.7	1:4.6	1:3.5
Ratio width protomerite: width deutomerite .....	1:1.6	1:1.8	1:1.5
Satellite:			
Total length sporont.....	100	105	98
Length protomerite .....	20	20	20
Length deutomerite .....	80	85	78
Width protomerite .....	37	40	40
Width deutomerite .....	55	56	60
Ratio length protomerite: length deutomerite .....	1:1.5	1:1.4	1:1.5
Ratio width protomerite: width deutomerite .....	1:1.5	1:1.4	1:1.5
Cyst, outer diameter.....	77	90	86
Inner diameter .....	63	74	66
Thickness transparent layer around cyst..	7	8	10

*Frenzelina olivia* nov. spec.

Figures 8 to 10

The host of this species is the small littoral spider crab, *Libinia dubia*, which is abundant along the shores of Long Island Sound and its inlets. The parasite is found in the upper part of the intestine. It generally occurs in moderate numbers (10 to 100) but rarely an infection of 1,000 or more is encountered.

The sporonts are biassociative and average  $80\mu$  in length and  $35\mu$  in width. They are ellipsoidal in shape, rounded in front and rather blunt posteriorly. The protomerite is hemispherical, very slightly constricted at the septum. It is about one fifth the total length of the sporont and slightly papillate in the adults (Fig. 8); the younger solitary sporonts possess a conspicuous papilla (Fig. 10). The deutomerite is but little wider than the protomerite (1:1.2); in solitary individuals it is more broadly rounded at the posterior end than in those which are attached. The endocyte of the mature sporonts is dark brown and very dense in the deutomerite; the protomerite is less dense and tan in color. At the anterior end of the protomerite is an orange colored disc. The nucleus is visible only in immature specimens. It is spherical and generally contains one large karyosome.

Movement of progression is rapid and continues only for intervals of about two seconds.

Cysts are spherical, dark brown in color, and from 45 to  $60\mu$  in diameter including the enveloping wall. They occur in the posterior third of the intestine. No sections were made of the host intestine.

This species is placed in the genus *Frenzelina* because (1) it is biassociative, (2) there is a papillated conspicuously differentiated apical area in the protomerite, (3) it is very similar to *Frenzelina delphinia* in form and location and both occur in hosts from the same habitat; (4) it is parasitic in the intestine of a marine crustacean.

The larger spider crab, *Libinia emarginata*, which is found in deeper water and seldom comes near the shore, has been examined repeatedly for gregarines, but none have been found to date. Other crabs procured from oyster boats, and which were dredged in the Sound and Harbor, have not yielded gregarines. These include *Neopanope texana sayi*, *Carcinides maenas*, *Pagurus bernhardus* and *Pagurus longicarpus*; and *Chloridella empusa* from the mud flats of the Inner Harbor. From the south side of the island, the following crabs have been examined and none found to be infected with gregarines: *Emerita talpoida*, *Callinectes sapidus*, *Ovalipes ocellatus*, *Ocypoda albicans*, and an undetermined species of *Orchestia*.

It seems possible that only littoral marine crustacea are infested with gregarines and that spores are eaten along with shore vegetation,

grasses and tide water algae, and are swept away by the tides or are noninfective when they reach the water.

A table of measurements, in microns, follows:

Total length association.....	218	195	150	127
Primate:				
Length sporont .....	100	85	80	65
Length protomerite .....	20	20	14	14
Length deutomerite .....	80	65	66	51
Width protomerite .....	35	38	30	30
Width deutomerite .....	43	48	45	36
Ratio length protomerite: total				
length sporont .....	1:5	1:4.2	1:5.7	1:4.6
Ratio width protomerite: width				
deutomerite .....	1:1.2	1:1.3	1:1.5	1:1.2
Satellite:				
Length sporont .....	118	110	70	62
Length protomerite .....	25	14	10	10
Length deutomerite .....	83	96	60	52
Width protomerite .....	36	39	22	22
Width deutomerite .....	36	50	28	30
Ratio length protomerite: total				
length sporont .....	1:5	1:8	1:7	1:6.2
Ratio width protomerite: width				
deutomerite .....	1:1	1:1.3	1:1.3	1:1.4

*Frenzelina nigrofusca* nov. spec.

Figures 11 to 14

Two species of fiddler crabs, *Uca pugnax* and *U. pugilator*, which live together at the roots of the eel grass, were found to be infected with a species of gregarine. About 30 per cent. of the hundred or more crabs examined were parasitized and the infection was very moderate; in only rare instances was the number of parasites present greater than fifteen.

The sporonts were solitary, none being associative as is characteristic of this genus. The body is broadly ovoidal, less than twice as long as wide (Fig. 11) and is often nearly rectangular in shape with rounded corners (Fig. 12). Sporonts average  $100\mu$  in length and  $65\mu$  in width. The protomerite is hemispherical and very slightly constricted at the septum; it is about one third the length of the whole sporont. There is a minute papilla at the anterior end; this papilla is large and conspicuous in the trophozoite (Fig. 13). The deutomerite is of approximately the same width as the protomerite and twice as long. It is very broadly rounded or often flattened posteriorly.

The endocyte is very dense and appears dark brown or black in transmitted light. It is but little less dense in the protomerite than in the deutomerite and in starved animals becomes tan in color. The nucleus is not visible in the live sporonts. The sarcocyte is relatively thick, especially over the anterior end of the protomerite. The nucleus

is small and spherical and contains one or two minute karyosomes. Uniform gliding movement was observed at a relatively slow rate. The cysts are very dense and are spherical. Spores were not seen.

This species is placed in the genus *Frenzelina* for two reasons: (1) the protomerite possesses at its apex a small papillated and conspicuously colored disk, this papilla being well developed in the trophozoite, (2) the gregarine infects the intestine of a marine crustacean. While no associative sporonts were seen, the species is very probably associative from its affinities.

A table of measurements of a few specimens is appended here, all dimensions being given in microns:

Total length sporont.....	72	82	100	120	125
Length protomerite .....	20	22	22	31	31
Length deutomerite .....	52	60	78	89	94
Width protomerite .....	39	40	70	50	75
Width deutomerite .....	37	40	75	75	65
Ratio length protomerite: total length .....	1:3.6	1:3.9	1:4.5	1:4	1:4
Ratio width protomerite: width deutomerite .....	1:1	1:1	1:1	1:1.5	1:2.1
Diameter nucleus .....	1	1.1	1.5		

#### SUMMARY

1. Three new Gregarine parasites of the genus *Frenzelina* are described from marine Crustacea.
2. Parasites belonging to this genus have not hitherto been reported from the United States.
3. A new definitive character for this genus has been determined upon sectioning the host intestine, namely, the fact that the parasites are intercellular.

#### REFERENCE CITED

- Léger, L., and Duboscq, O. 1909. Etudes sur la sexualité chez les Grégarines. Arch. Prot., 17: 19-134.



## EXPLANATION OF PLATE

*Frenzelina delphinia* nov. spec.

1. An association of two sporonts from lumen of intestine of *Talorchestia longicornis*; *a*, protomerite of sporont, *b*, deutomerite, *c*, primite, *d*, satellite.
2. Another association with satellite much younger than primite.
3. Cross section of portion of intestine of *Talorchestia longicornis* showing intercellular development of parasite; *a*, oblique section of trophozoite embedded in epithelium; *b*, *c*, sections of sporonts lying free in lumen but near the walls and surrounded by mucus.
4. Interlocking device by which satellite of association is attached to primite.
5. Association revolving in an early stage of cyst formation.
6. Cross section of portion of host intestinal epithelium showing embedded trophozoite in longitudinal section.
7. Cyst soon after completion, showing transparent cyst wall and two sporonts still distinctly outlined.

*Frenzelina olivia* nov. spec.

8. Mature association from lumen of intestine of *Libinia dubia*.
9. Immature sporont from same location.
10. Young sporont free in intestinal lumen, showing papilla at anterior end of protomerite. Magnification greater than in Figures 8 and 9.

*Frenzelina nigrofusca* nov. spec.

- 11, 12. Adult sporonts from lumen of intestine of *Uca pugnax*.
13. Trophozoite from lumen of host, showing papilla.
14. Sporonts with posterior half of body contracted, indicating that considerable movement of the body is possible.

# PLATE

